AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Bacteriology®

COMMENTARY

Stress Reduction, Bacterial Style

Susan Gottesman

Laboratory of Molecular Biology, NCI, Center for Cancer Research, Bethesda, Maryland, USA

ABSTRACT Bacteria have robust responses to a variety of stresses. In particular, bacteria like *Escherichia coli* have multiple cell envelope stress responses, and generally we evaluate what these responses are doing by the repair systems they induce. However, probably at least as important in interpreting what is being sensed as stress are the genes that these stress systems downregulate, directly or indirectly. This is discussed here for the Cpx and sigma E systems of *E. coli*.

KEYWORDS Cpx, Hfq

All is for the best in the best of all possible (bacterial) worlds—as long as evolution has done its job, preparing bacteria for every challenge. What we refer to as "stress responses" are the changes in gene expression as bacteria adapt to changing or suboptimal environments. We can most easily define these responses by the regulator that controls the change—whether it is a specialized sigma factor, a two-component system, or some other transcriptional regulator. In the lab, we can induce these systems by a variety of treatments or genetic tricks, and the combination of the inducing condition and the changes in gene expression leads us to define a regulon as a particular stress response—to Fe starvation (Fur regulon [1]), to DNA damage (SOS response [reviewed in reference 2]), or in the cases discussed here, to perturbations in establishing and maintaining the outer surface of the cell, the periplasm, and the inner membrane (IM), monitored in part by the cell surface stress sigma factor sigma E (also called RpoE), mediating outer membrane homeostasis, and the Cpx two-component system, dealing with IM homeostasis (reviewed in references 3 and 4). However, what is it that the cell is really sensing as stress? A study by T. Raivio and coworkers in this issue (5) suggests a key role for IM protein complexes in the Cpx envelope stress response, providing a new view of why the cell has evolved the Cpx regulon. The results in many ways provide parallels to the sigma E envelope stress response.

To allow us to study a given stress response, we first need to have identified the key regulator necessary for the response; usually, a mutant deficient in this will provide the first definition of the regulon. Second, we need a way to induce the response at will. Once those two components are available, defining the downstream output is reasonably straightforward. Genetic methods, in particular, transcriptional fusions, first defined many stress responses (6), but now transcriptome sequencing can be used to identify the range of transcriptional changes, both direct and indirect. This can be refined to direct targets through approaches like chromatin immunoprecipitation. However, it is becoming increasingly clear that this information does not always mean we understand what the stress response is responding to and fixing. If we understand what targets the induced enzymes might repair, that should provide some information on what damage induces the response. However, even in a well-studied bacterium like *Escherichia coli*, there will be multiple genes of unknown or poorly defined function induced in response to a given stress, or the induced genes may represent a fairly indirect part of the initial stress. In general, however, we assume that genes that are induced in response to a stress are protective. What about those that are downregulated when the stress response is induced? I will argue here that these genes are frequently the key to understanding what a stress response is really about.

Accepted manuscript posted online 31 July 2017

Citation Gottesman S. 2017. Stress reduction, bacterial style. J Bacteriol 199:e00433-17. https://doi.org/10.1128/JB.00433-17.

Editor Victor J. DiRita, Michigan State University

Address correspondence to Gottesms@helix.nih.gov.

For the article discussed, see https://doi.org/10.1128/JB.00153-17.

Our thinking about envelope stress responses starts here with what is known about the sigma E response (reviewed in reference 3). A significant class of outer membrane proteins (OMPs) contain, at their C terminus, sequences that are hidden when the proteins are properly inserted into the outer membrane; when these proteins are misfolded or not properly trafficked to the outer membrane, these sequences are available to interact with and activate the IM protease DegS (7). DegS then cleaves the anti-sigma factor RseA, leading to activation of the sigma E response (8). The proteins that are induced as part of the sigma E response include chaperones to help properly fold OMPs, the transport machinery that places beta-barrel proteins in the membrane, and periplasmic proteases to help rid the cell of irreversibly misfolded proteins (9). Sigma factors, which direct RNA polymerase to promoters, do not directly carry out negative regulation. However, there are clearly multiple genes downregulated when sigma E is induced, including many OMPs (9). Thus, as part of the sigma E response, the synthesis of the proteins that, when mistargeted, cause the stress in the first place is reduced. Much, if not all, of this downregulation is via sigma E-dependent small RNAs (sRNAs) (10, 11). These sRNAs pair with mRNAs, dependent on the RNA chaperone Hfq; pairing leads to, in the cases discussed here, translational repression and mRNA degradation. Absence of these and other sRNAs, or of Hfq, leads to constitutive induction of the sigma E response (11–13), reinforcing the idea that if synthesis of OMPs is not negatively regulated by sRNAs, the flux to the outer membrane may be too great for cells to handle.

The work presented in this issue by Raivio and coworkers (5), combined with recent work on the roles of the CpxQ sRNA (14, 15), suggests that for Cpx as well, downregulation of the clients of the Cpx-induced proteins defines critical components that the regulatory response protects. As with the sigma E response, the induced arm of the Cpx response includes chaperones and protein foldases to promote correct protein folding or to help degrade proteins that are beyond chaperone-induced repair (16).

What functions, then, are downregulated when Cpx is induced? The Raivio group found, in earlier microarray studies, strong downregulation of two multiprotein IM complexes, NADH dehydrogenase and cytochrome bo_3 (17). In contrast to sigma factors, the CpxR response regulator can carry out direct repression, and in fact, this is the case for the *nuo* operon, encoding NADH dehydrogenase, and the *cyo* genes, encoding cytochrome bo_3. Why are they downregulated? If it is true that the targets of downregulation are proteins that cause the stress in the first place, deletion of these genes might relieve the stress; that is what Raivio and coworkers find. Thus, in a *cpxR* mutant, when the cell cannot properly mount a Cpx response, cells are killed by treatment with sublethal doses of the antibiotic amikacin or high pH, but deletion of either the *nuo* or the *cyo* gene relieves this lethality (5). Strikingly, deletion of these gene clusters also significantly lowers the induction of a Cpx reporter normally induced as cells enter stationary phase. As with a number of other membrane proteins, overexpression is sufficient to induce Cpx. These results all suggest that proper folding and assembly of these IM respiratory complexes are major targets for the Cpx response and that problems with their assembly or function mediate, at some level, the induction of the response.

In addition to direct repression by Cpx, other genes are downregulated indirectly by sRNAs when Cpx is induced. Most notable is CpxQ, an Hfq-binding sRNA encoded at the 3′ end of the *cpxP* gene, one of the most highly induced promoters of the Cpx response (14, 15). Overexpression of CpxQ downregulated multiple mRNAs in *Salmonella*; the mRNAs were enriched for those encoding proteins with IM or periplasmic localization. This included the *nhaB* mRNA, encoding a sodium-proton antiporter previously shown to be downregulated when Cpx is induced (17). Another target, Skp, is a periplasmic chaperone suggested to be capable of mistargeting OMPs into the IM, collapsing the proton motive force (15).

The downregulated genes identified in all of these studies are consistent with the idea that the Cpx system's major function is to monitor and control something about the status of the IM. What is actually being monitored? One possibility, as noted by

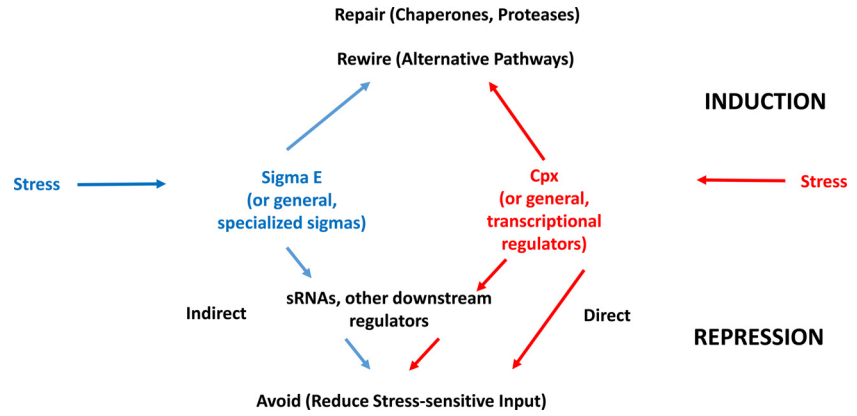

FIG 1 Shown is a highly simplified version of two types of stress response, exemplified by the sigma E (blue) and Cpx (red) responses to cell envelope stress. In both cases, and for many other stresses, induced genes of the regulons encode functions involved in damage repair or avoidance, while repressed genes, whether directly or indirectly (for instance, via sRNAs) repressed, encode the key processes the stress response must protect.

Guest et al. (5), is that multisubunit complexes of this sort need help assembling and inserting, and when they do not, the Cpx system can help. For instance, while the transcription of the enzymes of the respiratory complex is unchanged in the absence of the Cpx two-component system, aerobic respiration is significantly decreased. Deleting the genes that encode either of two major IM protein complexes might then suppress the need for CpxR for resistance to high pH or to the antibiotic amikacin, as found by Guest et al., by reducing the need to assemble one major complex. A somewhat more specific (but not mutually exclusive) model suggests that Cpx is, in fact, a response to changes in the energy status of the cell, reflected in somewhat decreased respiration when Cpx is activated (and presumably downregulating the respiratory complexes encoded by the *nuo* and *cyo* genes). This model would easily explain why deleting either of two operons in a given pathway sometimes leads to a similar resistance to stress in cells with *cpxR* deleted and would predict that deletion of genes encoding other proteins involved in respiration, whether large complexes or not, might be similarly protective. Guest et al. argue that, overall, this explanation is less likely.

Figure 1 outlines the general circuitry that I suggest is relevant to thinking about most stress systems. Stress leads to induction of the regulon; regulon members will include increased expression of the factors that repair the stress damage or avoid it, possibly by using alternative metabolic pathways. Regulon members that are repressed, however, may highlight what is the most basic source of the stress. Such downregulation can be direct (as for Cpx repression of *nuo* and *cyo* in the work by Guest et al.) or indirect, via intermediate regulators, in particular, sRNAs. While the discussion here is focused on two envelope stress systems, there is every reason to expect the lessons learned from these to be universal.

ACKNOWLEDGMENT

The writing of this commentary was supported by the intramural program of the NIH, National Cancer Institute, Center for Cancer Research.

REFERENCES

1. Massé E, Salvail H, Desnoyers G, Arguin M. 2007. Small RNAs controlling iron metabolism. Curr Opin Microbiol 10:140–145. https://doi.org/10.1016/j.mib.2007.03.013.
2. Simmons LA, Foti JJ, Cohen SE, Walker GC. 2008. The SOS regulatory network. EcoSal Plus 2013 https://doi.org/10.1128/ecosalplus.5.4.3.
3. Grabowicz M, Silhavy TJ. 2017. Envelope stress responses: an interconnected safety net. Trends Cell Biol 42:232–242. https://doi.org/10.1016/j.tibs.2016.10.002.
4. Raivio TL. 2014. Everything old is new again: an update on current research on the Cpx envelope stress response. Biochim Biophys Acta 1843:1529–1541. https://doi.org/10.1016/j.bbamcr.2013.10.018.
5. Guest RL, Wang J, Wong JL, Raivio TL. 31 July 2017. A bacterial stress response regulates expression of respiratory protein complexes to control envelope stress adaptation. J Bacteriol https://doi.org/10.1128/JB.00153-17.
6. Kenyon CJ, Walker GC. 1980. DNA-damaging agents stimulate gene

expression at specific loci in *Escherichia coli*. Proc Natl Acad Sci U S A 77:2819–2823. https://doi.org/10.1073/pnas.77.5.2819.
7. Walsh NP, Alba BM, Bose B, Gross CA, Sauer RT. 2003. OMP peptide signals initiate the envelope-stress response by activating DegS protease via relief of inhibition mediated by its PDZ domain. Cell 113:61–71. https://doi.org/10.1016/S0092-8674(03)00203-4.
8. Ades SE, Connolly LE, Alba BM, Gross CA. 1999. The *Escherichia coli* sigma(E)-dependent extracytoplasmic stress response is controlled by the regulated proteolysis of an anti-sigma factor. Genes Dev 13:2449–2461. https://doi.org/10.1101/gad.13.18.2449.
9. Rhodius VA, Suh WC, Nonaka G, West J, Gross CA. 2006. Conserved and variable functions of the sigmaE stress response in related genomes. PLoS Biol 4:e2. https://doi.org/10.1371/journal.pbio.0040002.
10. Guisbert E, Rhodius VA, Ahuja N, Witkin E, Gross CA. 2007. Hfq modulates the sigmaE-mediated envelope stress response and the sigma32-mediated cytoplasmic stress response in *Escherichia coli*. J Bacteriol 189:1963–1973. https://doi.org/10.1128/JB.01243-06.
11. Gogol EB, Rhodius VA, Papenfort K, Vogel J, Gross CA. 2011. Small RNAs endow a transcriptional activator with essential repressor functions for single-tier control of the global stress regulon. Proc Natl Acad Sci U S A 108:12875–12880. https://doi.org/10.1073/pnas.1109379108.
12. Thompson KM, Rhodius VA, Gottesman S. 2007. σ^E regulates and is regulated by a small RNA in *Escherichia coli*. J Bacteriol 189:4243–4256. https://doi.org/10.1128/JB.00020-07.
13. Bossi L, Maloriol D, Figueroa-Bossi N. 2008. Porin biogenesis activates the sigma(E) response in Salmonella hfq mutants. Biochimie 90:1539–1544. https://doi.org/10.1016/j.biochi.2008.06.001.
14. Chao Y, Vogel J. 2016. A 3′ UTR-derived small RNA provides the regulatory noncoding arm of the inner membrane stress response. Mol Cell 61:352–363. https://doi.org/10.1016/j.molcel.2015.12.023.
15. Grabowicz M, Koren D, Silhavy TJ. 2016. The CpxQ sRNA negatively regulates Skp to prevent mistargeting of β-barrel outer membrane proteins into the cytoplasmic membrane. mBio 7:e00312-16. https://doi.org/10.1128/mBio.00312-16.
16. Vogt SL, Raivio TL. 2012. Just scratching the surface: an expanding view of the Cpx envelope stress response. FEMS Microbiol Lett 326:2–11. https://doi.org/10.1111/j.1574-6968.2011.02406.x.
17. Raivio TL, Leblanc SKD, Price NL. 2013. The *Escherichia coli* Cpx envelope stress response regulates genes of diverse function that impact antibiotic resistance and membrane integrity. J Bacteriol 195:2755–2767. https://doi.org/10.1128/JB.00105-13.